

### **Amendments to the Claims**

This listing of claims will replace all prior versions, and listings, of claims in the application:

#### **Listing of Claims:**

1. (Currently Amended) An *in vitro* assay method to detect a physical or chemical change involving a chemical or biological species which comprises the steps of:
  - a) performing an assay on a biological species using an assay reagent containing at least one NMR active nucleus, said assay reagent being one of i) introduced as an initial reagent, ii) formed in situ during the assay, and iii) formed as a product of the assay, and
  - b) hyperpolarising at least one NMR active nucleus of the assay reagent; wherein steps (a) and (b) are performed simultaneously or sequentially in either order, and
  - c) analysing the assay reagent and/or the assay by NMR for a physical or chemical change in said biological species that is independent of the interaction of the biological species with the NMR active nucleus; and
  - d) optionally using the NMR data obtained in step c) to generate further assay result(s).
2. (Previously presented) The method of claim 1 wherein the NMR active nucleus comprises one of  $^{15}\text{N}$ ,  $^{19}\text{F}$ ,  $^{31}\text{P}$ ,  $^1\text{H}$ ,  $^{29}\text{Si}$  and  $^{13}\text{C}$ .
3. (Original) The method of claim 2, wherein the NMR active nucleus is  $^{15}\text{N}$  or  $^{13}\text{C}$ .
4. (Previously presented) The method of claim 1, wherein the assay reagent is a compound which contains an artificially high concentration of an NMR active nucleus.

5. (Currently amended) The method of claim 4, wherein the assay reagent contains an artificially high concentration of the NMR active nucleus in 1-10 up to 10 defined positions.
6. (Previously presented) The method of claim 1, wherein the assay reagent is an organic compound comprising one or more NMR active nuclei associated with a bond which is broken during the course of the assay.
7. (Original) The method of claim 6, wherein the assay reagent contains two or more NMR active nuclei and each NMR active nucleus produces a distinct NMR spectrum and when the assay method is performed, it results in changes to the chemical and/or physical environment of the nucleus and this is mirrored by spectral changes which can be monitored.
8. (Previously presented) The method of claim 1, wherein the assay reagent is analysed repeatedly in step c) at known time intervals so as to generate information about a change with time of the assay reagent.
9. (Previously presented) The method of claim 1, wherein the assay reagent is a Nucleotide, nucleotide analogue, polynucleotide, amino acid analogue, polypeptide or protein.
10. (Previously presented) The method of claim 1, wherein the assay is a nucleic acid hybridisation assay.
11. (Previously presented) The method of claim 1, wherein the assay is a binding assay.
12. (Previously presented) The method of claim 1, wherein the assay reagent is a compound specifically labelled with at least one NMR active nucleus and the assay reagent is administered to a micro-organism, macro-organism or cultured cells,

cellular metabolites or an excretion product of the assay reagent are hyperpolarised and analysed by nuclear magnetic resonance spectroscopy, nuclear magnetic resonance imaging or both.

13. (Previously presented) The method of claim 1, wherein the assay is a binding study performed using micro-organisms or cultured cells
14. (Currently amended) The method of claim 1 wherein ~~the hyperpolarisation transfer~~ said step (b) is repeated to enhance the signal-to-noise ratio.
15. (Previously presented) The method of claim 1 wherein the method exhibits a shortening effect as expressed by the improvement of signal-to-noise per unit time by a factor of 10 or more compared to said method being carried out without hyperpolarisation.
16. (Previously presented) The method of claim 1 where the hyperpolarisation of the NMR active nucleus of the assay reagent is carried out by polarisation transfer from a hyperpolarised noble gas, or a mixture of hyperpolarised noble gases.
17. (Original) The method of claim 16 wherein the noble gas is  $^{129}\text{Xe}$ .
18. (Original) The method of claim 16 wherein the noble gas is  $^3\text{He}$ .
19. (Previously presented) The method of claim 16 wherein the hyperpolarisation is transferred by a hyperpolarised noble gas in solution and wherein the viscosity of the solution is at least 1000 mPs.
20. (Previously presented) The method of claim 1 where the hyperpolarisation of the NMR active nucleus of the assay reagent is carried out by polarisation transfer at a temperature of 4.2 K or less in the presence of a magnetic field of at least 1 T.

21. (Previously presented) The method of claim 1 where the hyperpolarisation of the NMR active nucleus of the assay reagent is carried out by polarisation transfer using dynamic nuclear polarisation.
22. (Previously presented) The method of claim 1 where the hyperpolarisation of the NMR active nucleus of the assay reagent is carried out by para hydrogen induced polarisation.
23. (Previously presented) The method of claim 1 where the hyperpolarisation of the NMR active nucleus of the assay reagent is carried out with the spin refrigeration technique.
24. (Previously presented) The method of claim 1, wherein more than one assay is multiplexed and monitored by NMR spectroscopy and/or NMR imaging.
25. (Previously presented) The method of claim 1 wherein the assay is performed in a multiwell or multispot assay array.
26. (Previously presented) The method of claim 1 wherein step c) is performed by examining the assay reagent using both NMR spectroscopy to obtain more than one spectrum, and magnetic resonance imaging to obtain one or more discrete spectral location, and repeating the examination at least once so as to obtain quantitative information about kinetic or time-dependant alteration in chemistry, environment or structure of the assay reagent.
27. (Previously presented) The method of claim 1, wherein step c) is performed in an aerosol or flow-through device applied to aerosol droplets where the well, surface or container is used to contain the assay reagent.

28. (Currently amended) An *in vitro* assay kit ~~for carrying out the assay method as defined in claim 1~~ which comprises: one or more assay reagents each containing at least one NMR active nucleus, ~~contained in and~~ a well or vial or other container adapted to contain said one or more assay reagents and suitable for carrying out a step of hyperpolarizing said active nucleus of said one or more assay reagents ~~the hyperpolarisation of step (b) of claim 1.~~
29. (Currently amended) The *in vitro* kit of claim 28 wherein said well or vial or other container is suitable for carrying out a step of analyzing said one or more assay reagents by NMR ~~where the NMR analysis of step (c) is carried out in the same well, vial or container as the hyperpolarisation transfer is carried out.~~